The University of Chicago

FLORAN ANATOMY AND MORPHOLOGY OF ANEMOPSIS CALIFORNICA

A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY
1941

By
CHARLES H. QUIBELL

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FLORAL ANATOMY AND MORPHOLOGY OF ANEMOPSIS CALIFORNICA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 524

CHARLES H. QUIBELL

(WITH THIRTY-EIGHT FIGURES)

Introduction

This paper describes the vascular supply to and in the individual flower in the inflorescence and also the development of the ovule and megagametophyte. *Anemopsis californica* (Nutt.) H. and A. is a moisture demanding, alkali tolerant, perennial herb of the southwestern United States and northern Mexico, for which a new variety subglabra has been recently established by KELSO (7). The material used here is of that variety.

Anemopsis is a member of the Saururaceae, frequently included as a tribe in the Piperaceae. On the basis of anatomical considerations, ROUSSEAU (8) has reviewed the systematic treatment of the Piperales and concludes that *Saururus* is more closely related to *Piper* than the latter is to *Peperomia* of the Piperaceae. HOLM (3) has described the vegetative morphology and anatomy of the species, including the anatomy of the involucral and floral bracts. SCHMITZ (9) has described the development of the flower and vascular anatomy of several species of the Piperales but not of *Anemopsis*. JOHNSON (6), in a paper dealing with the relationships of the order, has reviewed the development of four genera already investigated and added data on four more, including *Anemopsis*. Of this he reports that "the functional megaspore is one out of two potential megaspores. . . . .A tapetum is formed and is persistent. . . . .The mature embryo-sac is a typical seven-nucleate one. . . . .The first division of the endosperm nucleus is followed by a cell wall cutting the embryo-sac into an upper and a lower cell . . . . it is the upper one of these two primary cells that divides further to form a considerable mass of endosperm. The lower cell forms an elongated flask-shaped haustorium with but a single nucleus." JOHNSON does not elaborate these statements and his paper is not illustrated. HÄUSER (2) interprets JOHNSON'S statement with regard to two megaspores to mean that after the first division of the megaspore mother cell only the lower nucleus divides again and the megagametophyte is derived from the two megaspores resulting from such division. On the basis of the same report, SCHNARF (11) assumes tentatively that the megagametophytic development is of the *Scilla* type. The observations reported here confirm SCHNARF'S assumption. A 16-nucleate megagametophyte derived from four megaspores has

been reported for *Peperomia pellucida* (1, 5). The investigations stimulated by this discovery have been summarized by SCHNARF (10, 11). Although all the seventeen species of *Peperomia* which have been studied possess 16-nucleate megagametophytes, and other 16-nucleate megagametophytes have been discovered in several unrelated families, no other genus in the Piperales exhibits megagametophytes with more than the usual number of eight nuclei. These megagametophytes are derived, in different genera, from one, two, or four megaspores.

Material and methods

The material was collected at various stations near Fresno, but chiefly in a meadow along Kings River above Centerville, California. The inflorescences, or more frequently sections of them, were killed and fixed in a variety of fluids, of which an alcohol-formalin-acetic acid mixture proved most satisfactory. Heidenhain's haematoxylin-orange G combination was satisfactory.

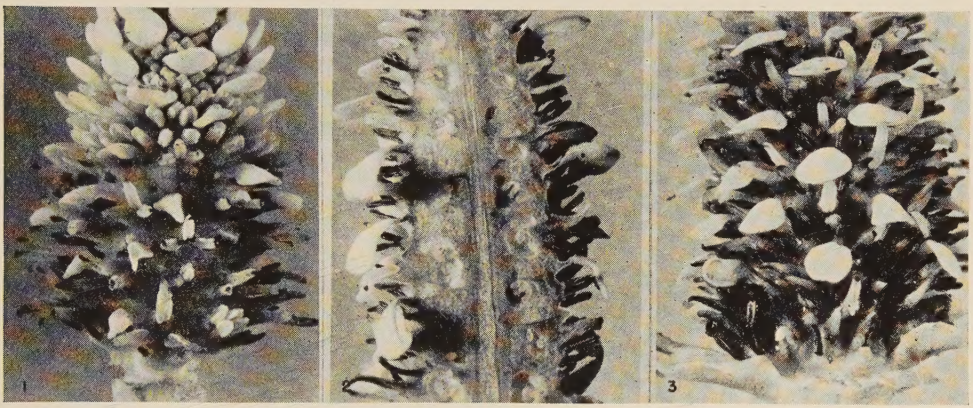
Observations

INFLORESCENCE AND FLOWER.—The thick horizontal rhizome is perennial, a rosette of leaves expanding from its apical bud each spring. From the axils of some of these leaves arise long stolons. Vegetative propagation seems to be the chief means of distribution, few seedlings having been observed. From the axils of other leaves rise single flowering stems whose apices are at first surrounded by the single cauline bract borne on these stems. The cylindrical or slightly conical primary inflorescence, pushed up out of this enveloping bract by the growth of its peduncle, is then protected by the whorl of 6–8 white involucral bracts which are turned up over it. When these spread out horizontally, the inflorescence is still covered for a short time by the small floral bracts, one subtending each of the numerous, spirally arranged flowers, except the basal ones just above the involucral bracts. The horizontal spreading of these bracts is acropetal (fig. 1).

The individual flowers, when thus uncovered, for a brief period show only the closely packed, turgid tips of the six yellow anthers. Subsequent growth spreads the anthers, uncovering three grooved stigmas, whose growth then brings their tips well above the anthers (fig. 1). The ovary has a single locule and three parietal placentae. It is completely inferior, so that the stigmas and the nearly sessile anthers project from the general surface of the inflorescence axis (fig. 2). Each of the three stigmas is grooved the full length of its inner face. These three grooves are united at the surface of the inflorescence axis to form an actual or potential pore leading into the locule. According to JEPSON (4) there are 6–10 ovules on each placenta or 18–30 ovules per flower. Counts of thirty flowers (excluding those near the apex of the inflorescence) distributed among six heads averaged 8.5 ovules per flower. The arrangement of two stamens opposite each carpel is shown in

figures 1 and 3. Two or three leaves arise from a meristem in the axil of the cauline bract and sometimes one or two secondary inflorescences arise in their axils.

Vascular supply of individual flower.—The vascular bundles of the axis of the inflorescence are arranged in an irregular ring. The vascular supply of the individual flower is a single bundle (fig. 4 *X*) which, at the level of the next flower directly below, swings outward from the ring of bundles, rises parallel to it until the base of the flower is reached, and then is divided into seven bundles, all of which are turned outward around the locule (figs. 5–8). At a point level with the top of the locule six of these seven bundles undergo a series of divergences and



Figs. 1–3.—Fig. 1, lower three-fourths of inflorescence. Fig. 2, longisection showing ovaries surrounded by inflorescence axis. Fig. 3, similar to and somewhat older than inflorescence in fig. 1.

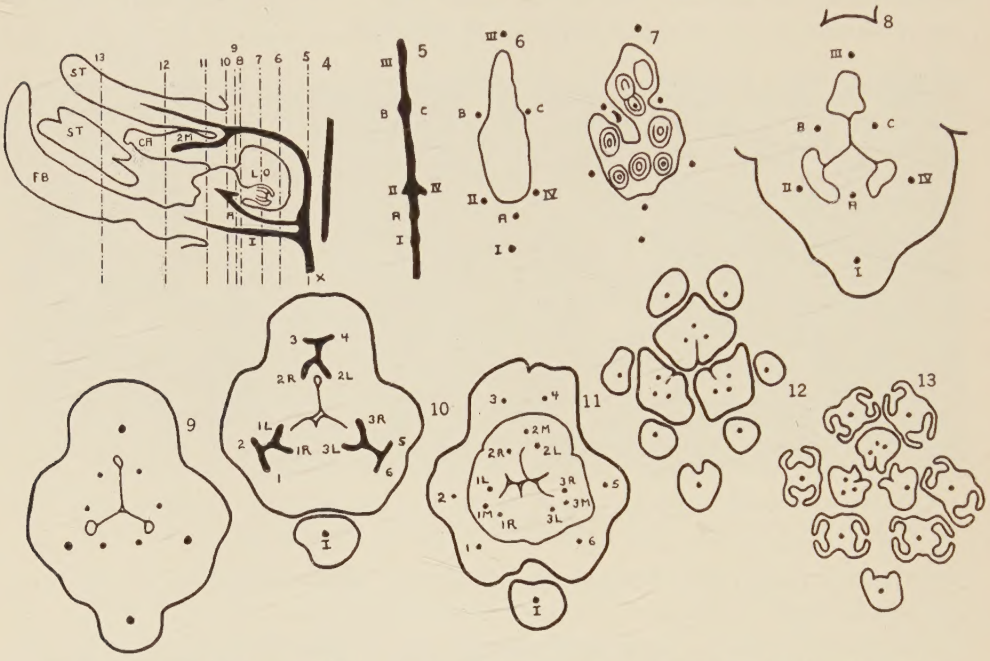
anastomoses (figs. 4, 9–11) which result in fifteen bundles supplying the filaments and styles (figs. 12, 13).

The bundle of the bract (*I*) is the first divergence from the main supply bundle, and without anastomosing with any other it provides the entire vascular supply for that organ. The lower placental bundle (*A*) is the next divergence, followed in turn by two paired divergences, the lower stamen bundles (*II*, *IV*) and the upper placental bundles (*B*, *C*), and by the upper stamen bundle (*III*) which extends out over the upper side of the locule.

The placentae are parietal and extend little more than half way down the locule wall (fig. 2). Above them (level with the top of the locule) the placental bundles split in two, diverge right and left at sharp angles, and then ascend as lateral bundles in the two corresponding styles. The stamen bundles, one opposite each carpel, extend around the locule to a point somewhat above its top, are split trichotomously, and diverge at sharp angles. One branch extends inward and is the central bundle in the style, while the other two spread right and left and extend into the two stamens associated with the carpel. The central bundle of each style,

at the point where it is turned upward into it, is usually connected by horizontal branches with the two stylar laterals.

There are occasional variations from this pattern. The basal flowers in the inflorescence lack floral bracts. No instance of variation in the number or arrangement of the six placental and stamen bundles has been observed, but variations in the fifteen divergences from them are comparatively frequent. A relatively large proportion of flowers lack one or more stamens or a style. Short abortive



FIGS. 4–13.—Fig. 4, longisection of flower bud in inflorescence still inclosed by involucral bracts. Figs. 5–13, cross sections of flower in bloom, at levels corresponding to those indicated by numbered lines on fig. 4. *FB*, floral bract; *ST*, stamen; *CA*, upper end of carpel; *LO*, locule and one ovule; *I*, floral bract bundle; *II*, *III*, *IV*, stamen supply bundles; *A*, *B*, *C*, placental bundles; *1*, *2*, *3*, *4*, *5*, *6*, stamen bundles; *1R*, *1M*, *1L*, *2R*, *2M*, etc., right middle, and left stylar bundles of carpels *1*, *2*, and *3*.

styles, lacking stigmas, have their middle bundles diverged from the corresponding stamen bundle, but they do not have lateral bundles, which if diverged at all from the corresponding placental bundles form only short vestigial traces which do not extend into the style base. All instances of abortive stamens showed at least some anther development and vascular bundles. Possibly those abortive stamens which consist of a short sharp-tipped filament, with no vestige of anther development, would have no vascular bundle.

A doubling of all the lateral stylar bundles was observed in one of the three flowers examined in each of two inflorescences. The extra bundles were short,

derived by splitting of the divergences from the placental bundles, and terminated at about the level of the anastomoses between the main and lateral bundles in or just below the stylar base. They are not involved in this anastomosis. The anastomoses between main and lateral stylar bundles are frequently incomplete, but in no flower observed were they absent in all styles. Occasionally a placental bundle is diverged into three instead of two, and the extra one is connected with the base of a middle stylar bundle.

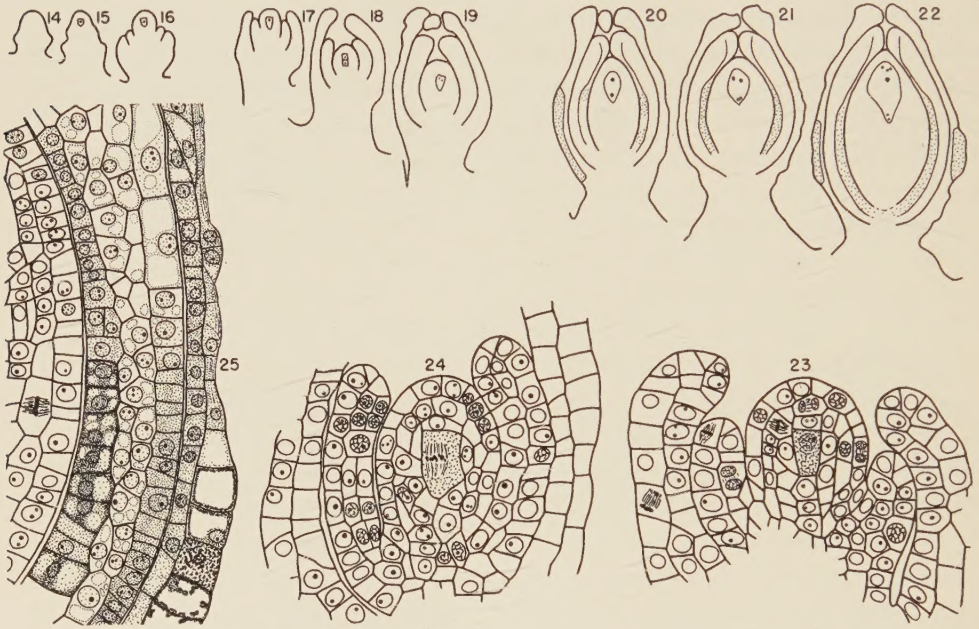
Integuments.—The outer integument arises first, followed so quickly by the inner that the latter for a brief period may overgrow the former (figs. 14–16). Until the four megaspore nuclei have been formed, the growth rate of the outer integument exceeds that of the nucellus, so that a large space is developed above the latter (figs. 17, 18). As a result of change in relative growth rates during the prolonged 2-nucleate phase of the megagametophyte, this space is occupied by the nucellus and the rim of the inner integument (figs. 19, 20), and subsequently the growth rates of the integuments and nucellus keep pace with each other (figs. 21, 22). Both integuments are two cell layers in thickness in the beginning (fig. 23). In the outer integument the number usually does not increase except at its base and apex, when development of the embryo is well advanced. In the inner integument a third layer results from periclinal divisions in the inner layer, beginning about the time the megaspore mother cell divides (fig. 24). A fourth layer is more or less completely formed owing to doubling of the middle layer, when the megagametophyte is 8-nucleate (fig. 25). The largest cells of the ovule occur in the outer layer of the inner integument opposite to and above the megagametophyte. These cells are three or more times as long as broad, even as early as the end of the 2-nucleate phase (figs. 20, 25). During the latter half of this phase the cells of the inner layer, opposite and below the megagametophyte, grow much larger in both dimensions than those in the upper part. Beginning in isolated cells, the protoplasts of this portion of the layer all take a deep stain by the end of the 4-nucleate phase. In post-fertilization stages even the thinner portion of the layer over the top of the nucellus stains in this manner (figs. 20–22, 25).

In the outer integument the cells of the outer layer become much shrunken and stain weakly, so that their end walls are difficult to distinguish; but there are occasional short vertical rows of much enlarged cells which protrude from the surface of the ovule and later stain deeply like those of the inner layer of the inner integument (figs. 20, 22, 25).

Nucellus.—The apex of the nucellus, above the point of origin of the inner integument, is at first small and rounded (fig. 15), but by the time the rim of the outer integument passes beyond this apex the median longitudinal section of the nucellus is a truncated oval or ellipse (fig. 17). It shows little subsequent change in size or shape until after the megaspore mother cell divides, although in the

meantime the integuments and the ovule base have grown considerably (figs. 17, 18). During the 2-nucleate phase of the megagametophyte the shape of the nucellus, in longitudinal section, becomes a considerably less truncated ellipse (figs. 18–20). Its growth in length is most pronounced below the megagametophyte, with the result that the latter exchanges its original central position for one in the upper half of the nucellus.

The megaspore mother cell and two parietal cells arise from a hypodermal primary sporogenous cell. These three cells are recognizable in some ovules when



FIGS. 14–25.—Successive stages in ovule development. Megaspore mother cell present in fig. 15 and in prophase of meiosis in figs. 16 and 17; first meiotic division complete in fig. 18; 2-nucleate stages of megagametophyte in figs. 19 and 20, 4- and 7-nucleate stages in figs. 21 and 22. Figs. 23–25, stages in integument development from prophase of meiosis (fig. 23) to 8-nucleate phase of megagametophyte (fig. 25).

the inner integument is initiated (figs. 26, 27). During the prophase of the first meiotic division the outer parietal cell frequently undergoes anticlinal division. A periclinal division of this cell or any division of the inner parietal is rare (figs. 23, 24, 28, 29). Subsequent encroachment of the 2-nucleate megagametophyte upon the parietal cells is steadily balanced by their growth and division tangentially until the megagametophyte nuclei divide, after which frequently only one nucellar cell intervenes between megagametophyte and epidermis (figs. 32–38).

MEGASPORE MOTHER CELL.—This cell begins the protracted prophase of the

FIGS. 26–38. —Fig. 26, megaspore mother cell and primary parietal cell; inner integument just starting. Fig. 27, primary parietal cell divided; megaspore mother cell still differentiated only slightly, except in size. Fig. 28, megaspore mother cell in prophase of meiosis. Fig. 29, same in synizesis. Fig. 30, first meiotic division completed. Fig. 31, four megaspores in two cells after considerable growth has occurred in chalazal pair. Fig. 32, late stage in degeneration of micropylar megaspores. Fig. 33, 2-nucleate megagametophyte following resorption of micropylar megaspores. Fig. 34, nuclei at poles and in position for division; megagametophyte and nuclei slightly larger than average. Fig. 35, anaphase of division in 2-nucleate megagametophyte; axis of spindles at right angles to that of megagametophyte and to each other; chalazal division figure complete; one set of chromosomes of micropylar figure present in next section. Fig. 36, 4-nucleate megagametophyte. Fig. 37, polar nuclei about to fuse; egg to left. Fig. 38, 7-nucleate megagametophyte; egg nearer to observer than synergid, and its cytoplasm very diffuse. Antipodals represent extreme of preservation for this stage. Upper antipodal drawn in from second section.

first meiotic division before the rim of the outer integument passes beyond that of the inner (figs. 16, 28). The axis of the spindle for this division in the cases observed is parallel to the long axis of the ovule (fig. 24). Of the resulting cells the chalazal one is usually slightly larger (fig. 30). Division of the two daughter nuclei follows quickly. In two of the four instances observed there is a slight lag in the division of the micropylar nucleus, while in the others division is simultaneous. In all the spindles the axes were either oblique or across the cells and at various angles with each other. Walls were not formed, nor was there indication of a cell plate. The result is four megaspore nuclei in two cells,—the chalazal one, as subsequent events show, being the beginning of the 2-nucleate phase of the megagametophyte (fig. 31).

MEGAGAMETOPHYTE.—Marked size differences between the two pairs of nuclei become apparent quickly, since the micropylar nuclei do not grow following division, while the chalazal pair does (fig. 31). Growth of the megagametophyte begins soon after disappearance of the spindles, and in its first phase is entirely at the expense of the micropylar cell and the two megaspore nuclei it contains, so that at the end of this phase the megagametophyte has attained the size and shape of the megaspore mother cell at its initial division (figs. 30–32). The nuclei in the micropylar cell do not change in their staining reactions until at, or just before, their obvious structural degeneration begins. When this occurs the protoplast shrinks, with the cytoplasm clinging to the wall at several points. As the cell is further crushed, the nuclei become somewhat smaller. Then the cell and its contents are reduced to a dark staining cap and finally completely resorbed (figs. 32, 33).

The two nuclei of the megagametophyte lie close together almost as soon as formed, one above the other, at or near the center of the cell. Vacuoles appear above and below the nuclei without disturbing their position (figs. 31, 32). With complete disappearance of the micropylar cell, the nuclei frequently are shifted to a side-by-side position, but in the majority of instances the two vacuoles persist. When there is only one vacuole it may occupy either end of the megagametophyte (fig. 33). The megagametophyte grows steadily at the expense of the nucellus, until it has become about double the dimensions of the megaspore mother cell. The nuclei then move apart and take up positions nearer the poles, with a vacuole between them (fig. 34). Division follows quickly, and in the three instances observed the angles of the spindle axes with the axis of the ovule and with each other vary (fig. 35).

The 4-nucleate phase of the megagametophyte is relatively brief (fig. 36). In some inflorescences the chalazal end of the megagametophytes extends down nearly to the midpoint of the nucellus, while in others it reaches no farther than a fourth or a third the nucellar length. The four megagametophyte nuclei are in two

pairs, each pair imbedded in a dense, dome-shaped mass of cytoplasm at opposite ends of a large central vacuole. When first formed all four nuclei are equal in size, but while there is considerable increase in the diameters of the micropylar pair the chalazal pair does not grow (fig. 36). Division is simultaneous in the three examples observed; and in one 4-nucleate megagametophyte whose nuclei were just about to pass into the metaphase and in three very young 8-nucleate megagametophytes, all the nuclei are in the same stage. The 8-nucleate phase at first reflects the situation of the 4-nucleate phase regarding the size differences of the nuclei and the rather dense and restricted cytoplasmic masses at the poles. Each nucleus quickly organizes its mass of cytoplasm, in which there begins a characteristic type of differentiation. In the chalazal group the cytoplasm often becomes located chiefly at the sides of the nucleus and is somewhat strung out. The chalazal polar nucleus becomes readily distinguishable when the three antipodal nuclei shrink and are more chromatic, while it at first shows little change in size or appearance. Since degeneration of the antipodals is sometimes delayed or is not simultaneous, there are instances in which the chalazal polar nucleus cannot be definitely identified for a time.

The four micropylar nuclei are easily distinguished by the time the chalazal polar nucleus can be recognized with certainty. The polar nucleus derived from the micropylar end is always slightly lower down in the group relative to the vertical axis of the megagametophyte, but it is never quite separated from the others. The positions of the egg and synergid nuclei relative to the longitudinal axis of the megagametophyte are variable, but the synergid nuclei are readily recognized by their large and rather dense masses of cytoplasm, which occupy—or shortly come to occupy—the major portion of the micropylar end of the megagametophyte and become less dense or vacuolate at the chalazal margins. The egg nucleus, crowded against the side, has a much smaller mass of cytoplasm in which a very large vacuole usually develops, either laterally or toward the micropyle. Its nucleole is usually appreciably smaller than those of the synergid nuclei (fig. 37).

Before the polar nuclei fuse the chalazal one has increased in size to equal that of the micropylar (fig. 37). Fusion always occurs in the chalazal half of the megagametophyte, usually rather close to the antipodals. The endosperm nucleus is large and sometimes migrates to the micropylar end of the megagametophyte (fig. 38). By this time, the egg apparatus is well differentiated. The antipodals have usually degenerated considerably; one or more of them may have disappeared completely. Figure 38 illustrates the extreme of preservation of antipodals at this stage; more often the cytoplasm of those persisting has collapsed against the chalazal end of the megagametophyte, and the nuclear outlines—or even the nucleoles—are difficult or impossible to distinguish. Fertilization has not been observed.

Summary

1. The flower of *Anemopsis californica*, subtended by a small floral bract, consists of six stamens, three carpels, and an ovary completely buried in the inflorescence axis.

2. The vascular supply of the individual flower and its bract originates in a single bundle of the ring of bundles in the inflorescence axis. This bundle splits up into seven: the bract bundle and three stamen bundles alternating with three placental ones.

3. Above the ovary the stamen and placental bundles branch and anastomose in such a way as to provide one bundle for each stamen and three for each style.

4. The ovule is orthotropous, with two integuments and an eventually massive nucellus. The outer integument is initiated first. Both integuments exhibit certain differentiations in their cell layers.

5. The megaspore mother cell, which with two parietals arises from a hypodermal cell, divides twice, but a wall is formed only after the first division.

6. The pair of megaspores in the upper cell degenerate and the megametophyte is formed after two more nuclear divisions of the two megaspores in the lower cell.

7. The polar nuclei fuse in the chalazal end of the megagametophyte before fertilization.

8. The antipodals have usually disappeared or are degenerating before fertilization.

The subject of this investigation was suggested by Dr. G. M. SMITH of Stanford University. Encouragement and direction were given during the progress of the work by members of the department of botany of the University of Chicago.

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